

IN THE CLAIMS:

The following will replace all prior claim listings. Please amend the claims as shown.

LISTING OF THE CLAIMS

Claim 1. (Currently Amended) A process for cleaving a polypeptide comprising the steps of:

(1) providing a polypeptide comprising arginine or lysine at the P1 position of a desired cleavage site, an amino acid other than aspartic acid, glutamic acid or proline at the P1' position, and a single basic amino acid, or two or three consecutive basic amino acids situated at any site in the amino acid sequence from the P10 position to the P3 position or from the P3' position to the P5' position (with the proviso that a single basic amino acid is not situated at the P6 or P4);
and

(2) cleaving the polypeptide with OmpT protease ~~A polypeptide cleavage method characterized in that arginine or lysine is at the P1 position of a desired cleavage site in a polypeptide, an amino acid other than aspartic acid, glutamic acid or proline is at the P1' position, a single basic amino acid or two or three consecutive basic amino acids are situated at any site in the amino acid sequence from the P10 position to the P3 position or from the P3' position to the P5' position (with the proviso that a single basic amino acid is not situated at the P6 or P4 position), and OmpT protease is used to cleave the desired cleavage site in said polypeptide.~~

Claim 2. (Currently Amended) A process according to claim 1, wherein the polypeptide is a fusion protein comprising a protecting peptide and a target peptide wherein the C-terminus of the protecting peptide is the P1 position and the N-terminus of the protecting peptide is the P1' position, and wherein the fusion protein is produced by expressing a gene encoding the fusion protein in host cells in the step (1), and the fusion protein is cleaved with OmpT protease so as to liberate the target peptide in the step (2) ~~A method for producing a target peptide characterized by obtaining a target peptide from a fusion protein, the cleavage site of the~~

~~fusion protein being a desired cleavage site comprising a protecting peptide whose C-terminus is arginine or lysine, fused via the desired cleavage site with a target peptide whose N-terminus is an amino acid other than aspartic acid, glutamic acid or proline, wherein a single basic amino acid or two or three consecutive basic amino acids are situated at any site in the amino acid sequence from the P10 position to the P3 position or from the P3' position to the P5' position (with the proviso that in the case of a single basic amino acid, it is not situated at the P6 or P4 position), host cells are transformed with an expression plasmid having a gene coding for the fusion protein wherein said cleavage site is a cleavage site which is cleavable by OmpT protease, and said gene is expressed in said cells and is cleaved by said protease at said cleavage site.~~

Claim 3. (Previously Presented) The method of claim 1 wherein, if a site which is not desired to be cleaved by OmpT protease is present in the polypeptide or the fusion protein, cleavage at said site is inhibited by situating an acidic amino acid at the P3 position of said site.

Claim 4. (Previously Presented) The method of claim 1, which comprises situating two or three consecutive basic amino acids between the P10 and P3 positions of the desired cleavage site in the polypeptide or fusion protein.

Claim 5. (Original) The method of claim 4, which comprises situating three consecutive basic amino acids between the P5 and P3 positions of the desired cleavage site in the polypeptide or fusion protein.

Claim 6. (Previously Presented) The method of claim 1, wherein the basic amino acids are arginine and/or lysine.

Claim 7. (Original) The method of 6, wherein the basic amino acids are arginine.

Claim 8. (Original) A polypeptide cleavage method wherein OmpT protease is used for cleavage at a desired cleavage site in the polypeptide, or a method for producing a target peptide which comprises cleavage at a desired cleavage site in a fusion protein, the method being characterized in that, if a site which is not desired to be cleaved by OmpT protease is present in said polypeptide or said fusion protein, cleavage at said site is inhibited by situating an acidic amino acid at the P3 position of said site.

Claim 9. (Previously Presented) The method of claim 8, wherein the acidic amino acid is aspartic acid.

Claim 10. (Previously Presented) The method of claim 1, wherein the amino acid sequence from the P5 to P1 positions of the desired cleavage site in the polypeptide or fusion protein is Arg-Arg-Ala-Arg (SEQ ID NO: 11).

Claim 11. (Previously Presented) The method of claim 1, wherein the amino acid sequence from the P7 to P1 positions of the desired cleavage site in the polypeptide or fusion protein is Asp-Ala-Arg-Arg-Arg-Ala-Arg (SEQ ID NO: 12).

Claim 12. (Original) A polypeptide cleavage method characterized by cleaving a desired cleavage site of a polypeptide using an OmpT protease 97th amino acid variant wherein the 97th amino acid from the N-terminus of the OmpT protease is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid or histidine.

Claim 13. (Original) A polypeptide cleavage method characterized in that, when the P1 position of the desired cleavage site in the polypeptide is arginine or lysine and the P1' position is an amino acid other than arginine or lysine, the desired cleavage site of said polypeptide is cleaved using an OmpT protease 97th amino acid variant wherein the 97th amino acid from the N-terminus of the OmpT protease is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid or histidine.

Claim 14. (Original) A polypeptide cleavage method characterized in that the P1 position of the desired cleavage site in the polypeptide is arginine or lysine, the P1' position is an amino acid other than arginine or lysine, a single basic amino acid or two or three consecutive basic amino acids are situated at any site in the amino acid sequence from the P10 position to the P3 position or from the P3' position to the P5' position, and the desired cleavage site of said polypeptide is cleaved using an OmpT protease 97th amino acid variant wherein the 97th amino acid from the N-terminus of the OmpT protease is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid or histidine.

Claim 15. (Original) A method for producing a target peptide, characterized by transforming host cells with an expression plasmid having a gene coding for a fusion protein comprising a target peptide fused with a protecting peptide via a desired cleavage site that can be cleaved by an OmpT protease 97th amino acid variant wherein the 97th amino acid from the N-terminus of the OmpT protease is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid or histidine, expressing said gene in said cells, and obtaining the target peptide from the fusion protein by cleavage with said protease at said cleavage site.

Claim 16. (Original) A method for producing a target peptide, characterized by transforming host cells with an expression plasmid having a gene coding for a fusion protein comprising a protecting peptide whose C-terminus is arginine or lysine fused with a target peptide whose N-terminus is an amino acid other than arginine or lysine, via a desired cleavage site that can be cleaved by an OmpT protease 97th amino acid variant wherein the 97th amino acid from the N-terminus of the OmpT protease is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid or histidine, expressing said gene in said cells, and obtaining the target peptide from the fusion protein by cleavage with said protease at said cleavage site.

Claim 17. (Original) A method for producing a target peptide, characterized by transforming host cells with an expression plasmid having a gene coding for a fusion protein wherein a single basic amino acid or two or three consecutive basic amino acids are situated at any site in the amino acid sequence from the P10 position to the P3 position or from the P3' position to the P5' position at a desired cleavage site of a fusion protein comprising a protecting peptide whose C-terminus is arginine or lysine fused with a target peptide whose N-terminus is an amino acid other than arginine or lysine, via the cleavage site, and said desired cleavage site is a cleavage site that can be cleaved by an OmpT protease 97th amino acid variant wherein the 97th amino acid from the N-terminus of the OmpT protease is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid or histidine, expressing said gene in said cells, and obtaining the target peptide from the fusion protein by cleavage with said protease at said cleavage site.

Claim 18. (Previously Presented) The method of claim 12 wherein, if a site which is not desired to be cleaved by the OmpT protease 97th amino acid variant is present in the polypeptide or fusion protein, cleavage at said site is inhibited by situating an acidic amino acid at the P3 position of said site.

Claim 19. (Previously Presented) The method of claim 12, which comprises situating two or three consecutive basic amino acids between the P10 and P3 positions of the desired cleavage site in the polypeptide or fusion protein.

Claim 20. (Original) The method of claim 19, which comprises situating three consecutive basic amino acids between the P5 and P3 positions of the desired cleavage site in the polypeptide or fusion protein.

Claim 21. (Previously Presented) The method of claim 14, wherein the basic amino acids are arginine and/or lysine.

Claim 22. (Original) The method of claim 21, wherein the basic amino acids are arginine.

Claim 23. (Original) A polypeptide cleavage method wherein an OmpT protease 97th amino acid variant is used for cleavage at a desired cleavage site in the polypeptide, or a method for producing a target peptide which comprises cleavage at a desired cleavage site in a fusion protein, the method being characterized in that, if a site which is not desired to be cleaved by the OmpT protease 97th amino acid variant is present in said polypeptide or said fusion protein, cleavage at said site is inhibited by situating an acidic amino acid at the P3 position of said site.

Claim 24. (Previously Presented) The method of claim 18, wherein the acidic amino acid is aspartic acid.

Claim 25. (Previously Presented) The method of claim 12, wherein the amino acid sequence from the P5 to P1 positions of the desired cleavage site in the polypeptide or fusion protein is Arg-Arg-Ala-Arg (SEQ ID NO: 11).

Claim 26. (Previously Presented) The method of claim 12, wherein the amino acid sequence from the P7 to P1 positions of the desired cleavage site in the polypeptide or fusion protein is Asp-Ala-Arg-Arg-Arg-Ala-Arg (SEQ ID NO: 12).

Claim 27. (Previously Presented) The method of claim 12, wherein the 97th amino acid from the N-terminus of the OmpT protease is leucine, methionine or histidine.

Claim 28. (Previously Presented) The method of claim 12, wherein the P1' position of the desired cleavage site of the polypeptide or fusion protein or the N-terminus of the target peptide is serine or alanine, and the 97th amino acid of the OmpT protease 97th amino acid variant used is leucine.

Claim 29. (Previously Presented) The method of claim 12, wherein the P1' position of the desired cleavage site of the polypeptide or fusion protein or the N-terminus of the target peptide is phenylalanine, alanine, serine, cysteine or tyrosine, and the 97th amino acid of the OmpT protease 97th amino acid variant used is methionine.

Claim 30. (Previously Presented) The method of claim 12, wherein the P1' position of the desired cleavage site of the polypeptide or fusion protein or the N-terminus of the target peptide is alanine, valine, isoleucine, methionine, serine, threonine, cysteine or asparagine, and the 97th amino acid of the OmpT protease 97th amino acid variant used is histidine.

Claim 31. (Previously Presented) The method of claim 2, wherein the target peptide is a peptide composed of between 22 and 45 amino acid residues.

Claim 32. (Original) The method of claim 31, wherein the target peptide is adrenocorticotrophic hormone (1-24), motilin or calcitonin precursor.

Claim 33. (Previously Presented) The method of claim 2, wherein the host cells are *E. coli*.

Claim 34. (Previously Presented) The method of claim 1, which comprises using as the cleaving protease bacterial cells expressing a gene coding for OmpT protease or an OmpT protease 97th amino acid variant wherein the 97th amino acid from the N-terminus of OmpT protease is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid or histidine.

Claim 35. (Previously Presented) The method of claim 1, which comprises co-expressing a gene coding for OmpT protease or an OmpT protease 97th amino acid variant wherein the 97th amino acid from the N-terminus of OmpT protease is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid or

histidine, and a gene coding for a polypeptide or fusion protein whose cleavage by said protease is desired.